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PCT WELTORGANISATION FÜR GEISTIGES EIGENTUM
Internationales Büro

INTERNATIONALE ANMELDUNG VERÖFFENTLICHT NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT)

(51) Internationale Patentklassifikation 6: C07H 19/10, 19/20, C07F 9/572, C12Q 1/68, C07H 21/00 — A1

(11) Internationale Veröffentlichungsnummer: WO 95/04747

(43) Internationales Veröffentlichungsdatum: 16. Februar 1995 (16.02.95)

(21) Internationales Aktenzeichen: PCT/EP94/02541

(22) Internationales Anmeldedatum: 30. Juli 1994 (30.07.94)

(30) Prioritätsdaten:
P 43 26 466.2 6. August 1993 (06.08.93) DE

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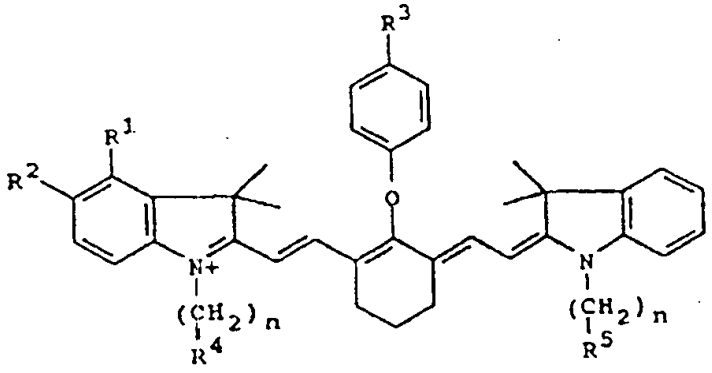
(81) Bestimmungsstaaten: AU, CA, FI, JP, KR, NO, NZ, US, europäisches Patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Veröffentlicht
Mit internationalem Recherchenbericht

(54) Title: INFRA-RED DYE-LABELLED NUCLEOTIDES AND THEIR USE IN NUCLEIC ACID DETECTION

(54) Bezeichnung: INFRAROT-FARBSTOFF-MARKIERTE NUCLEOTIDE UND IHRE VERWENDUNG IN DER NUCLEINSÄURE-DETEKTION



(I)

(57) Abstract

Nucleoside-5' triphosphates and phosphoramidites bearing in the base section or on the phosphorus atom a radical absorbent in the long wavelength, preferably a carbocyanine group of the general formula (I), in which R^1 and R^2 are hydrogen or together form a phenyl radical; R^3 is hydrogen if the bond with the nucleotide passes via the R^4 position, or an -NCHS- group if the bond with the nucleotide passes via the R^3 position; R^4 together with R^5 or R^5 alone represent an alkylsulphonyl group with n between 3 to 5 or R^4 is an -NHCS- group with a number of 3 to 8. The invention also relates to the use of the compounds for labelling, detecting and sequencing nucleic acids.

1.Stufe: 2',3'-didesoxy-uridin-5'-triphosphat

Das Derivat wurde ausgehend von kommerziell erhältlichem 2',3'-Didesoxy-cytidin-5'-triphosphat (Boehringer Mannheim) durch Desaminierung mit $NaNO_2$/Essigsäure über das instabile Diazonium-Derivat synthetisiert.

2.Stufe: 5-(3-aminoallyl)-2',3'-didesoxy-uridin-5'-triphosphat

Die Verbindung wurde analog Beispiel 1 nach Langer et al. über das 5-Mercuri-Derivat des 2',3'-didesoxy-UTP hergestellt.

3.Stufe: "IRD-ddUTP"

Das Didesoxy-Derivat wurde entsprechend Beispiel 2 durch Umsetzung des 5-Aminoallyl-ddUTP mit dem entsprechenden Isothiocyanat erhalten

Die spektralen Daten entsprechen denen der 2'-Desoxy-Verbindung aus Beispiel 3

Beispiel 7:

Anhydro-10,12-propylen-3,3,3',3'-tetramethyl-1,1'-bis (3-sulfopropyl)-indotricarbocyanin-11-[(4-ethoxy)phenoxy-O-(2-cyanoethyl)-N,N-diisopropyl-phosphoramidit

In einem 50 ml Rundkolben werden 425 mg Anhydro-11-(4-hydroxyethyl)phenoxy-10,12-propylen-3,3,3',3'-tetramethyl-1,1'-bis (3-sulfopropyl)-indotricarbocyanin-hydroxid in Form des Na-Salzes (0,5 mmol) in 5 ml trockenem Acetonitril gelöst und dazu 0,275 ml Ethyl-diisopropylamin (1,6 mmol) gegeben. Anschließend tropft man unter Stickstoff und Rühren 0,125 ml Chlor-ß-cyanoethoxy-N,N-diisopropylamino-phosphan innerhalb von ca. 3 Min. ein. Man rührt weitere 30 Min. bei RT, fügt dann ca. 10 ml wässrige, 5%ige $NaHCO_3$-Lösung zu und extrahiert daraufhin 2x mit je ca. 10 ml Dichlormethan. Die vereinigten organischen Phasen werden über Na-Sulfat getrocknet, das Lösungsmittel abdestilliert und der Rückstand an Kieselgel mit dem Laufmittel Dichlormethan/Ethylacetat/Triethylamin 45:45:10 chromatographiert.
Die Ausbeute beträgt 480 mg = 88,7 % d. Th.

DC (Kieselgel, Fließmittel wie o. a.) R_f = 0,4
^{31}P-NMR (d_6DMSO) : 149 und 153 ppm (2 Diastereomere)

INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/EP 94/02541

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07H19/10 C07H19/20 C07F9/572 C12Q1/68 C07H21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07F C07H C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 527 433 (MILES INC) 17 February 1993 see page 4, line 18 - line 43	1
A	EP,A,0 359 225 (E.I. DU PONT DE NEMOURS AND COMPANY) 21 March 1990 see page 9	13-15
A	WO,A,90 03383 (THE UNITED STATES OF AMERICA) 5 April 1990 see claims; figure 1	13-15

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 November 1994	16. 11. 94
Name and mailing address of the ISA	Authorized officer

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	'JOURNAL OF ORGANIC CHEMISTRY OF THE USSR. (ZHURNAL ORGANICHESKOI KHIMII), vol.57, 1992, NEW YORK US pages 4578 - 4580 STREKOWSKI ET AL 'Substitution Reactions of a Nucleofugal Group in Heptamethine Cyanin Dyes. Synthesis of an Isocyanato Derivative for Labeling of Proteins with a Near-Infrared Chromophore' see the whole document	1,13

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte onal Application No
PCT/EP 94/02541

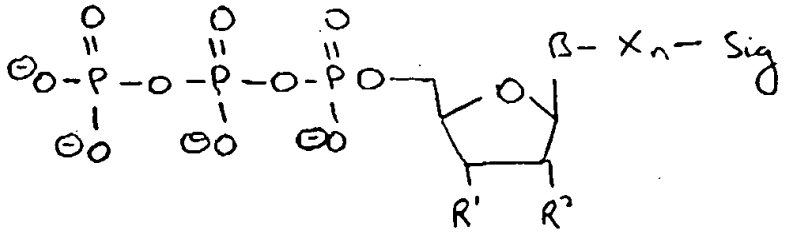
Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0527433	17-02-93	NONE		
EP-A-0359225	21-03-90	US-A-	4997928	05-03-91
		JP-A-	2174792	06-07-90
		US-A-	5262536	16-11-93
WO-A-9003383	05-04-90	AU-B-	618414	19-12-91
		AU-A-	4317989	18-04-90
		EP-A-	0436582	17-07-91
		JP-T-	4503403	18-06-92

CLAIMS FOR BOEHRINGER'S PATENT APPLICATION

PCT 95/04747

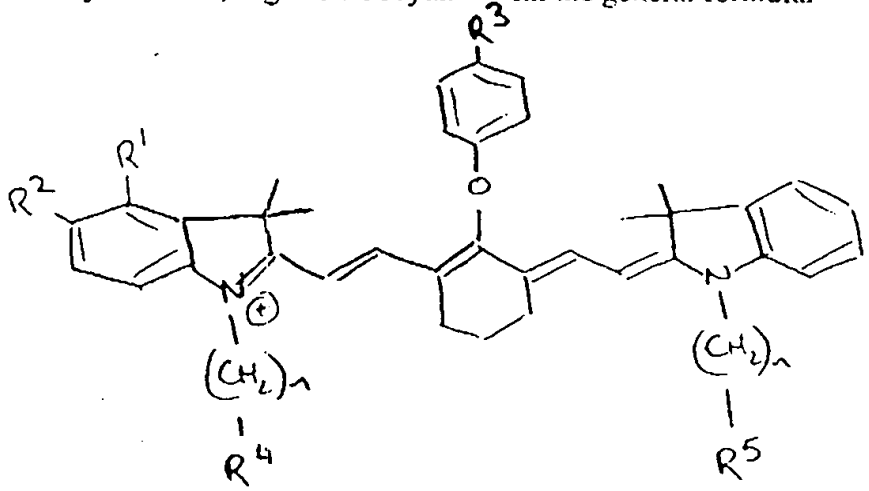
Patent claims

The Nucleosid-5'-triphosphate with the general formular



in which B stands for the heterocyclic bases Adenin, Guanin, Hypoxanthin, 7-Desaza-adenin, 7-Desaza-guanin, 7-Desaza-hypoxanthin, 7-Desaza-8-aza-adenin, 7-Desaza-8-aza-guanin, 7-Desaza-8-aza-hypoxanthin as well as Thymin, Cytosin and Uracil,
x is a connecting group with n= 4-20 atoms.
Sig. is a fluorescent molecule with exiting wavelength 650-800nm
and R^1 and R^2 are H and/or OH, respectively.

2. Compounds according to claim 1, with B as in 1, x a connecting group with preferably n=10-15, Sig a Carbocyanin with the general formular



and R_1 and R_2 are H or together one phenyl group.

R_3= H when the connection with the nucleotid is *via* the R_4 position, or -NHCS- when connection is *via* the R_3 position.,
R_4 and R_5 are each alkylsulfonyl with n = 3-5 or R_4 = -NHCS- with n = 3-8,
R5 = alkylsulfonyl with n =3-5 when the connection is *via* the R4 position.

3. Compounds according to claims 1 and 2 and their use as substrates for DNA-polymerases.

4. Compounds according to claims 1 and 2 and their use as substrates for RNA-polymerases.

5. The use of compounds according to claims 1 and 2 as marker of nucleonic acids.

6. The use of compounds according to claims 1 and 2 for the detction of nucleonic acid

7. The use of compounds according to claims 1 and 2 in DNA sequencing.

8. The use of compounds according to claims 1 and 2 in the *in situ*-hybridisation.

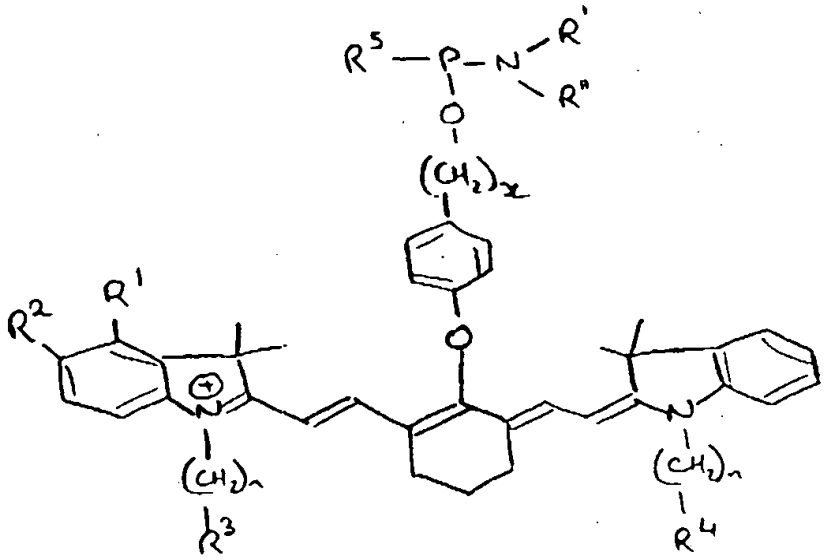
9. The use of compounds according to claim 8 when this hybridisation is performed on membranes.

10. The use of compounds according to claim 8 when this hybridisation is performed in solution.

11. The use of compounds according to claim 10 when this hybridisation is performed in solution on micro titer plates.

12. The use of compounds according to claim 6 when the detection of the marked hybrides is accomplished using laser diodes and detectors.

13. Compounds of the general formular



with R_1 and R_2 =H or together one phenyl group,
R_3 and R_4 each alkylsulonyl with n = 3-5, R_5 =methoxy or 2-cyanomethoxy and R' and R'' each ethyl or isopropyl and x = 1-10.

14. Use of the compounds according to claim 13 when these are used in oligo nucleotid synthesis in the Phosphorus-amidit-procedure.

15. Use of the compounds according to claim 14 for 5'-marking of oligo nucleotides.